Lasers in Surgery and Medicine 23:18–24 (1998)

Effects of Endogenous Absorption in Human Albumin Solder for Acute Laser Wound Closure

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Background and Objective: **Human albumin is currently being used as a biological solder in laser tissue welding. Experiments were performed to characterize the effects of differing albumin concentrations on wound closure when a 1.32 μm Nd:YAG laser is used to repair skin incisions.**
Materials and Methods: **In vivo comparison of acute tensile strength was made in full thickness porcine skin wounds using different solder concentrations. Histology of the repairs was also completed to evaluate the thermal denaturation of the tissue and solder. Transmission measurements were completed for nondenatured and denatured albumin solders. Finally, the real time denaturation pattern of different solder concentrations during laser irradiation was investigated.**
Results: **A tissue solder consisting of 50% albumin provides the greatest tensile strength for acute in vivo skin closure. The transmission measurements verify that the primary absorber of 1.32-μm laser light was the solder solvent (water). A significant decrease in power transmission occurs when the 25% albumin solder was denatured. The real time denaturation profiles demonstrate that 1.32-μm laser light denatures 25% albumin solder from the outer surface, while in 50% albumin solder, denaturation occurs from within the solder bulk. Wound histology corroborates the pattern of denaturation seen in vitro.**
Conclusion: **The combination of 1.32-μm laser light and 50% human albumin solder can be used to create a deep tissue weld resulting in higher acute repair tensile strength. This permits a deep to superficial closure of wounds, which may result in an optimal method of acute closure for full-thickness wounds. Lasers Surg. Med. 23: 18–24, 1998.** **© 1998 Wiley-Liss, Inc.**

Key words: skin; acute tensile strength; transmission; photon absorption; tissue welding

INTRODUCTION

The use of human albumin tissue solders in laser-assisted wound closure has met success in both ex and in vivo model systems [1]. In addition to significantly increasing the strength-of-tissue welds [2], human albumin solders have been shown to provide a rapid, fluid-tight wound clo-

Contract grant sponsor: National Institute of Diabetes and Digestive and Kidney Disease; Contract grant number: R41DK50505-01.

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Accepted 19 March 1998

sure. Further, these solders represent a form of protein sealant which is biodegradable, nonimmunogenic, and nonlithogenic in the urinary tract [3]. Thus, the use of laser welding techniques should have several advantages over conventional modes of wound closure, in both animal models [4–7] and in human surgical reconstruction [8].

The focus of laser welding research in the literature has been in animal models where the success of laser tissue welding is typically defined in terms of wound healing, leak point pressure, and fistula formation. Although there is substantial literature on the interaction of laser light with tissue, little information exists on the laser's interaction with human albumin tissue solders. To further optimize laser tissue welding with albumin solder, studies were undertaken to obtain a more precise understanding of the interaction between the laser light, the albumin solder, and living tissue.

Previous in vitro studies suggested that a 50% solution of human albumin solder combined with 1.32-μm laser light was optimal in regard to tissue weld strength [9]. In an effort to evaluate how changes in albumin concentration affect wound closure, we explored the interaction of laser light with human albumin in aqueous solution to determine the manner in which the laser absorption profile affects the denaturation of both the solder and tissue proteins—which results in the tissue weld. These studies look at the native absorption of the albumin solder, which differs from other solder systems that use exogenous chromophores. Therefore, these studies were based on measurements of the optical properties of the native solder, as relevant to the photothermal denaturation of albumin.

MATERIALS AND METHODS

Laser System

A 1.32-μm flash lamp-pumped Nd:YAG laser (Premier Laser Systems Inc., Irvine, CA) delivered via a 300-μm silica laser fiber was used in all of the experiments. The laser powers used for the individual studies are given below. Irradiation was continuous wave. This laser was chosen because the penetration depth of light at this wavelength (i.e., the 1/e-fold absorption length: 1.7 mm) [10] is comparable to the skin thickness (3 mm) for the porcine model used here. In addition, previous studies have demonstrated that this laser wavelength can be useful for full-thickness tissue weld in the skin [11].

Human Albumin Solder Materials

Albumin solders were prepared from commercially available (The New York Blood Center, New York, NY), highly purified, sterile, and viral-free lyophilized human serum albumin. A sterile aqueous stock solution of 50% (w/w) human albumin was prepared in distilled water [12]. Solders of lower concentration (<25%) were prepared via dilution of the stock 50% albumin solution using distilled water and sterile technique.

In Vivo Study of Acute Wound Tensile Strength

An animal study was undertaken to determine the optimal solder concentration for acute tensile strength in laser-assisted skin closure. A single Yucatan swine (20 kg) was used in a protocol approved by the Boston Children's Hospital Animal Care and Research Committee. The pig received humane care in compliance with the *Principles of Care and Use of Laboratory Animals* [13].

The animal was anesthetized with Ketamine (20 mg/kg) IM, followed by general mask anesthesia with Halothane. The animal was shaved, prepared with 70% ethyl alcohol, and draped. Nine 2-cm, full-thickness skin incisions were made over a template on the dorsum of the pig with a #11 scalpel blade. Wounds were irrigated with 0.001% epinephrine to minimize bleeding. The dorsal skin was cleaned with 0.3% hydrogen peroxide to remove dried blood. Three 2-cm full-thickness skin incisions were closed using one of three tissue welding techniques: (1) without solder, (2) with 25% albumin solder, or (3) with 50% albumin solder. Approximately 50 μl of solder was inserted between the tissue edges using a 22-gauge blunt-tipped needle. The laser power was set to 2.5 watts. A wire spacer running past the fiber to the tissue was used for tactile feedback to maintain a 2-mm distance from the fiber tip to the tissue during welding. This arrangement resulted in a constant 1.0 mm laser spot on the tissue. A visual endpoint for tissue welding used by the surgeon was blanching and thermal coagulation of the solder. The wounds were closed at a rate of approximately 1 mm per second.

After the incisions were repaired, the dorsal skin was excised as a single flap and placed on a moist towel to prevent desiccation. The fully anesthetized pig was then euthanized. For each of the three repair modalities, the tissue flap was divided sharply over a template into six 1-cm × 4-cm tissue sections (each 2-cm incision divided into

two 1-cm strips). Tensile strength measurements were completed within 2 hours of harvest.

Five of the six specimens in each of the three groups were tested for tensile strength using a commercial tensiometer (Instron Corp., Model Mini 55, Canton, MA). The wounds were stressed to failure at a rate of 20 mm/min and maximum stress (in kiloPascals) was determined. The sixth specimen was fixed in 10% buffered formalin for histological analysis to determine the zone of thermal injury. The samples were paraffin-embedded and sectioned perpendicularly to the repaired wound in 7 micron-thick slices. Tissues were stained with hematoxylin and eosin (H & E) or Masson's Trichome.

In Vitro Studies

Effect of albumin concentration on transmittance. The percent transmittance of 1.32 μm light through 25% and 50% human albumin solders was determined using a near infrared spectrometer (Nicolet Magna-IR™ Spectrometer 550) with a 10-mm pathlength optical cell. In addition, a spectra was obtained for the albumin solders throughout the near infrared region of interest (1.0–2.5 mm).

Effect of albumin solder denaturation on laser power transmission. To characterize the optical properties that occur when denaturing different concentrations of albumin solder, the transmission of the 1.32-μm Nd:YAG laser was determined at concentrations of 25% and 50%, while the laser denatured the human albumin solder. An optical cell (1-mm pathlength) was filled with human albumin solder and irradiated at a distance of 2 mm, using a 300-mm fiber with 1 watt of laser power. These thin layers of solder are similar to what overlies the wound during laser welding. Since power transmission was being measured for both native and denatured albumin, the power was chosen so that the thin albumin layer was not denatured prior to completing the measurement. Power transmission was measured with a power meter (Ophirr, Model 30A). Laser power was recorded before and after addition of each albumin concentration to the cell, and monitored during the laser denaturation of each sample. A total of six samples was tested in each of the three concentration groups.

Pattern of solder denaturation during laser irradiations. Optical changes that occurred during laser denaturation of columns of 25% and 50% solder were studied in a glass cylinder model to determine whether the changes in absorption of 1.32-μm light noted in the transmission studies have relevance in the pattern of laser denaturation of a column of albumin solder. Two hundred μl of human albumin at concentrations of 25% and 50% were added to glass cylinders forming an albumin column of 4 mm (height) by 2 mm (diameter). This in vitro model was used in an attempt to mimic the closure of the full-thickness wounds in pigs. The base of the chamber was constructed of an optically black material with a high absorption of 1.32-μm laser light. The column was then irradiated from the top using a 300 mm optical fiber to deliver the 1.32-μm laser light (set at a power of 1 watt) for 1 minute. These exposure parameters were chosen so that the total energy delivery to the solder column would be the same as for the in vivo studies. The pattern of albumin denaturation was videotaped (Sony camcorder with macro lens) for analysis.

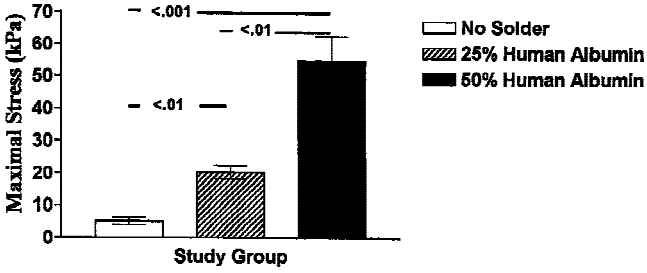

Fig. 1. Comparison of acute wound tensile strength for skin incisions repaired using the 1.32-μm laser without solder, and with 25% or 50% human albumin solder.

RESULTS

In Vivo Study of Acute Wound Tensile Strength

Acute tensile strength data for laser-repaired porcine skin wounds is given in Figure 1. Wounds laser welded with 25% ($P < 0.01$) and 50% ($P < 0.001$) albumin solder were significantly stronger with than wounds welded without solder. The laser repairs completed with the 50% albumin solder were significantly ($P < 0.01$) stronger than those completed with the 25% albumin solder.

Masson's TriChrome-stained sections from wounds welded with the 25% and 50% albumin solders are shown in Figure 2. For repairs completed using the 50% albumin solder, the wound edges and surrounding tissue and solder are denatured deep into the wound. In contrast, when a 25% albumin solder was used, generally less denaturation was seen along the wound edges deep in the tissue; and the solder was primarily dena-

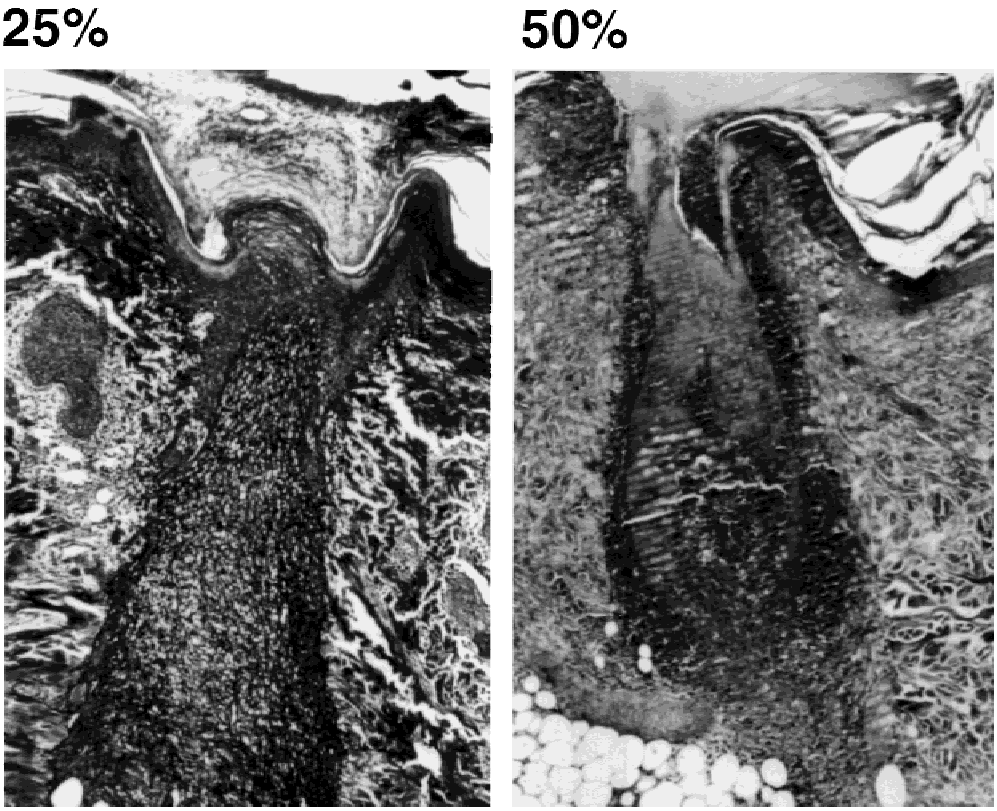

Fig. 2. Masson's TriChrome-stained tissue sections of a wound laser welded with 25% and 50% human albumin solder. Only superficial denaturation is seen in the repair completed with the 25% albumin solder, while denaturation of tissue and albumin solder deep in the wound is present when the 50% albumin solder is used.

tured at the surface of the tissue, resulting in a superficial castlike structure.

In Vitro Studies

Effect of albumin concentration on transmittance. The percent transmittance of 1.32-μm light through a 10-mm pathlength cell for 25% albumin and 50% albumin is given in Table I. The absorption length, l_α (1/e fold) calculated from this data is also given for the different albumin concentrations. The decreased absorption with increasing solder concentration clearly indicates that the primary absorber in the solder is the solvent (i.e., water). This was also verified by the spectrometer scan of the solder samples, which only showed significant absorbances at the expected water maxima [14] (e.g., at 1.44 and 1.94 mm).

Effect of albumin solder denaturation on laser power transmission. Laser transmission data through native (i.e., not denatured) and denatured 25%, and 50% human albumin are also given in Table I. The slight decrease in transmission measured for native solder can be explained by the reflections at the air glass interfaces of the sample cell. The laser denaturation of the albumin sample caused a decrease in laser transmission for both solder concentrations. In addition, the measured decrease in transmission was significantly greater for the denatured 25% ($P < 0.001$) albumin solder compared to the denatured 50% albumin solder. Since the decrease in transmission because of absorption is very small (>0.2% for the more highly absorbing, 25% albumin solution) for the 1 mm pathlength used, the large drops in transmission measured for the denatured solders can only be explained as increased scattering of the laser light by the denatured solders. Therefore, a scattering length l_s can be calculated for the denatured solder samples. These lengths are also given in Table I.

Pattern of solder denaturation during laser irradiation. Figure 3 is a composite graph created from video snapshots (arranged with Adobe Photoshop™ 3.0). Figure 3 shows denaturation that occurs when 1.32-μm laser light is directed through 200-μl columns of 25% and 50% human albumin. The top row represents images

TABLE 1. In Vitro Transmittance of Two Different Albumin Concentrations Before and After Denaturation*

Spectrometer results (Pathlength = 10 mm)		
Sample	Transmission (%)	l_α (mm)
25% solder		
Not denatured	20	6.2
50% solder		
Not denatured	30	8.2
Laser power transmission results (Pathlength = 1 mm)		
Sample	Transmission (%)	l_α (mm)
25% solder		
Not denatured	94 ± 3	—
Denatured	90 ± 3	9.5
50% solder		
Not denatured	95 ± 4	—
Denatured	30 ± 5	0.83

*The absorption length, l_α was measured using a spectrometer prior to albumin denaturation. The lower transmittance seen for the denatured samples is the result of increased scattering, given by a decrease in the scattering length, l_s.

taken after 3 seconds of laser irradiation, and the next two after 15 and 45 seconds, respectively. The ruler shows 1-mm intervals. The initial denaturation of the column of the 25% albumin solution occurs at the surface. When the albumin concentration is increased to 50%, the primary solder denaturation site occurs at the base of the column. With continued laser irradiation, the 25% albumin solder denaturation occurs laterally across the top of the column (i.e., subsurface albumin is not denatured). In contrast, 50% albumin solder denatures from the bottom of the column toward the surface.

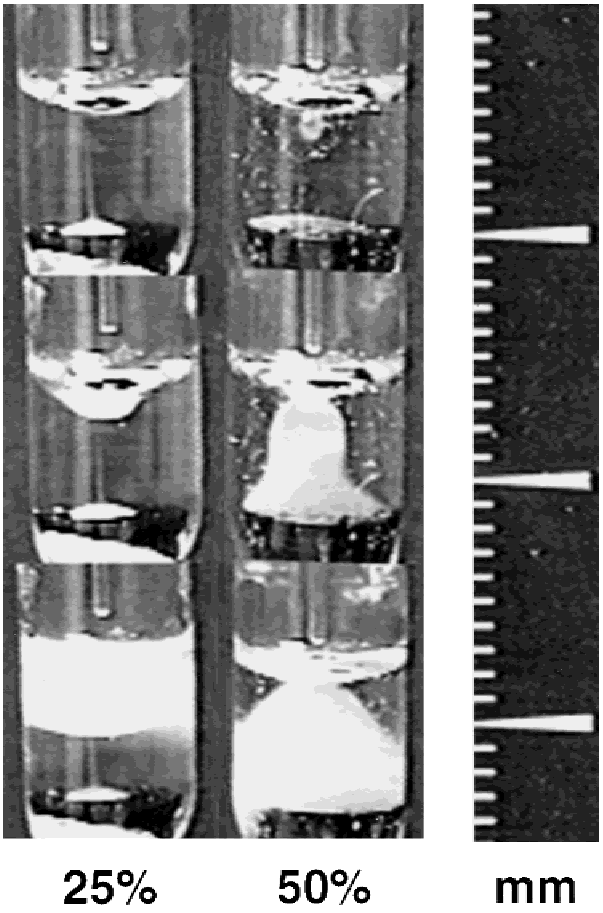

Fig. 3. Composite visual graph of the glass cylinder model where a column of albumin solder is laser irradiated and studied over time. The two columns shown are (left to right) 25% and 50% albumin solder denatured using a 1.32-μm laser. The scale at the far right gives depth in 1-mm intervals.

DISCUSSION

The thermal denaturation of complex proteins that occurs during tissue welding can range over a continuum from simple changes in tertiary structure to the breaking and formation of covalent bonds. Previous tissue welding studies using a 1.32-μm Nd:YAG laser to repair rat carotid arteries showed a loss of collagen perodicity and interdigitation of collagen fibrils without the formation of covalent bonds [15]. In addition, both visual changes and gross changes in consistency may occur with minimal heating as seen in the denaturation of egg albumin. Although it is not clear to what extent the albumin is denatured by 1.32-μm laser light or how the degree of denaturation translates into wound strength, the presence of albumin solder in a laser-welded wound at up to 2 weeks after closure indicates the laser denatures the solder to an insoluble form sufficient for solder preseverance.

These studies verified that the acute strength of a laser weld completed using a 1.32-micron laser is dependent upon the concentration of the albumin solder, and that a solder consisting of 50% albumin provides the strongest weld. Several possibilities may explain this result. A higher concentration of solder means a greater quantity of albumin molecules (per ml of solder) for binding with wound tissue and for the creation of the durable biological plug. In addition, a more highly concentrated solder may require less desiccation of water from the solder, which likely is an integral part of the denaturation process. Desiccation of water from the less-concentrated albumin solutions may also result in inclusions from bubble formation as the solvent (i.e., water) "boils" off.

The resulting air pockets will result in significant light scattering, as noted in the in vitro transmission (Table I) studies for the lower concentrated solder. Further, these inclusions may also decrease the bulk strength of the weld, compared to a weld consisting of a solid mass of denatured albumin.

The histologic results suggest an alternative explanation for the quantitative difference in acute wound strength for the two different solders. These results demonstrate that wounds closed using the 50% albumin solder result in both solder denaturation and evidence of laser irradiation deep in the base of the wound, while wounds closed with a 25% albumin solder show only superficial denaturation of both solder and tissue proteins. In addition, the histologic results suggest a deeper penetration of laser light in the wound closed with 50% albumin solder. This effect may result in a significantly larger weld volume (caused by the co-denaturation of the albumin and the tissue).

A concentration-dependent difference in the pattern of protein denaturation was also seen in the in vitro patterns of solder denaturation summarized in Figure 3. With the 25% albumin solder, laser energy absorption, photothermal conversion, and protein denaturation occur superficially in the column of albumin solder. The superficial denatured albumin then becomes more highly scattering (see also Table I), thereby preventing transmission of light to the base of the wound. The layer of denatured solder is thus a functional shield to both the deep solder and the interior wound borders. Alternately, Figure 3 shows that for the 50% albumin solder, 1.32-μm light passes freely through the column of solder and is absorbed at the black surface at the bottom of the column. As the albumin solder at the base denatures, backward scattering by the denatured solder increases the intensity of the light at the liquid/denatured solder interface resulting in a further denaturation of the overlying solder. As a result, with continued laser irradiation, the zone of denaturation in the column moves from the base of the column upward. We feel that it is the deep to superficial pattern of denaturation which has exciting implications in laser tissue welding.

In a laser tissue weld, where the laser wavelength-chromophore combination is such that the thermal energy is generated at the surface of the wound, decreased tensile strength of the weld may result. Our observations indicate that this decreased tensile strength may be in part because of low penetration of the laser energy into the wound, resulting in a superficial tissue weld. This is similar to the wound closures completed using a solder containing a highly absorbing exogenous chromophore (e.g., a dye) in conjunction with a laser tuned to the maxima of the chromophore. Changing the concentration of the albumin or the chromophore concentration may permit a degree of control for the photothermal conversion visa via the pattern of denaturation of the albumin solder.

This may also allow the surgeon to manipulate the tissue weld based on the wound's geometry and thickness. Whereas the laserlight passes relatively efficiently through the 50% albumin solder, it is more likely to be absorbed and converted into thermal energy at the albumin-tissue interface. This ability to seal a wound from deep to superficial may have novel clinical applications. For example, a 50% albumin solder, which is a viscous solution, may be injected into a fistula, and then denatured from deep to superficial by the laser. For these applications, the denatured albumin solder may provide an adherent biological plug. We (JMM, DPP) are currently investigating this application clinically.

Deep to superficial wound closure, as seen with the 1.32-μm laser/50% albumin solder combination, may also have implications for the addition of biologically active substances, such as growth factors, to the albumin solder. Since albumin is a primary carrier protein in serum, laser tissue welding with human albumin solder may not only represent a technique to create a watertight closure of wounds, but also a novel method to deliver biologically active substances to influence wound healing. One growth factor, TGF-β_1, when added to an albumin solder, has been shown to enhance wound strength during healing [16]. For these applications, delivery of growth factor through the depth of the wound requires a deep to superficial closure that can only be obtained with the 50% albumin solder.

In conclusion, our results indicate that the human albumin solder concentration affects the quality of acute laser repairs in skin incisions, as measured by acute tensile strength. Long-term healing effects were not studies here. By varying the albumin concentration in the solder, different patterns of in vitro solder denaturation results, which range from a surface cast like denaturation to a deep to superficial solder denaturation. Further optimization of the components of laser tissue welding should permit tissue repair with greater specificity and effectiveness, which will

ultimately impact the quality of wound closure using this novel technique.

ACKNOWLEDGMENTS

The authors wish to express gratitude to Joanne Curley, Ph.D. and the department of Anesthesiology at Children's Hospital in Boston for their assistance with the spectrophotometric analysis, and to the Department of Chemical Engineering at the Massachusetts Institute of Technology, Cambridge for the use of their spectrometer to determine the albumin transmission profile.

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